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Short Communication

Evaluation of the field efficacy of an avirulent live *Lawsonia intracellularis* vaccine in foals ☆

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ABSTRACT

Equine proliferative enteropathy caused by *Lawsonia intracellularis* is an emerging disease with as yet unaddressed preventative measures. The hypothesis of this study was that vaccination will prevent clinical and sub-clinical disease. Weanling Thoroughbreds ($n = 202$) from Central Kentucky were randomly assigned into two groups (vaccinated and non-vaccinated). Vaccinated foals received 30 mL of an avirulent, live *L. intracellularis* vaccine intra-rectally twice, 30 days apart. Foals were monitored for clinical disease, total solids and average weight gain until yearling age. There was an overall decreased disease incidence on the farms involved in the study that did not differ significantly between the groups. This decreased disease prevalence in the study population may be associated with the ongoing vaccine trial on these farms, as disease prevalence in Central Kentucky did not change in 2009 compared to 2008.

Equine proliferative enteropathy (EPE) caused by *Lawsonia intracellularis* induces epithelial hyperplasia of the jejunum and ileum that results in a protein-losing enteropathy (Lawson and Gebhart, 2000). EPE often presents as a sporadic disease, although herd outbreaks have been reported from Canada (Lavoie et al., 2000) and Central Kentucky in the USA (Frazer, 2008). The epidemiology of EPE is uncertain and although clinical disease of EPE has been well described in the veterinary literature sub-clinical disease has only been recently described (Pusterla et al., 2010). The purpose of the current study was to evaluate the protective effects of an intra-rectally administered avirulent, live vaccine of *L. intracellularis* in the prevention of EPE in foals residing on endemic farms and to document sub-clinical disease. We hypothesized that the vaccine would prevent clinical disease and that sub-clinically infected foals would display a lower concentration of total solids and a lower average daily bodyweight (BW) gain when compared to vaccinated herd mates.

The efficacy of the avirulent, live *L. intracellularis* vaccine was evaluated in a prospective, randomized field trial. Three Thoroughbred breeding farms in Central Kentucky were selected based on epidemiological data from 2008. These farms had at least 10% of their weanling population clinically affected by EPE in 2008. After owners' consent had been received, 202 healthy weanlings were randomly assigned into vaccinated ($n = 96$) or non-vaccinated ($n = 106$) groups. Foals were determined to be free of anti-*L. intracellularis* specific antibodies by immunoperoxidase monolayer assay (IPMA; Guedes et al., 2002). Vaccination was performed 30 days before the expected time of onset of clinical disease in September, 2009.

Vaccinated foals received 30 mL of the vaccine intra-rectally on day 0 and 30, a dose regimen which had been found to create a measurable immune response in weanlings (Pusterla et al., 2009a). Blood was collected on days 0, 30, 60, 90 and 120 for serological evaluation of *L. intracellularis* specific antibodies by IPMA and determination of total solid concentrations. One hundred and sixteen foals (58 vaccinated, 58 non-vaccinated) were weighed and had average daily BW gains calculated at days 0, 30, 60, 90 and 120. Vaccinated and control foals were housed together and were monitored daily for appearance of clinical signs of EPE up to yearling age. Feces from clinically affected animals were processed for nucleic acid purification and analyzed for the presence of *L. intracellularis* DNA by real-time polymerase chain reaction (PCR), as previously described (Pusterla et al., 2009b). To determine strain

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Table 1
Clinical and clinicopathological findings in the four clinical cases of EPE.

	Foal 1	Foal 2	Foal 3	Foal 4
Farm	Farm B	Farm C	Farm A	Farm A
Vaccine status	Non-vaccinated	Non-vaccinated	Non-vaccinated	Vaccinated
Clinical signs	Throat latch edema	None	Throat latch and sheath edema	Throat latch edema
Diagnostic test results	Fecal PCR: negative IPMA ⩾480	Fecal PCR: positive IPMA ⩾480	Fecal PCR: positive IPMA ⩾480	Fecal PCR: positive IPMA ⩾480
Lowest total blood solids (mg/dL) (Ref.: 5.8–8.7)	3.3	4.5	2.9	3.9
Lowest albumin (mg/dL) (Ref.: 2.7–4.2)	1.5	1.8	1.7	2.2
Recovery time (days)*	21	17	28	14

* Resolution of hypoproteinemia.

origin of *L. intracellularis* from PCR positive samples, Multiple-locus variable number tandem repeat analysis (VNTR) profiling was determined as previously reported (Beckler et al., 2004).

The primary outcome variable was occurrence of EPE, collected for analysis as a binary categorical variable. Cumulative incidence of EPE was determined for vaccinated and non-vaccinated groups and the difference was tested by Chi-square test for homogeneity. Seroconversion, defined as a positive titer at a 1:60 dilution based on results of IPMA, was then determined for all animals (Frazer, 2008). Foals were then re-categorized for data analysis based on these results. Vaccinated foals remained in the vaccinated group regardless of serological status. Non-vaccinated foals were categorized based on serological status as either seroconverted, due to natural exposure (IPMA titer ⩾60), or unaffected (IPMA titer <60). Data were analyzed by descriptive statistical analysis. The distributions of the continuous data for total solids and average daily BW gains were tested for normality. One-way repeated measure of ANOVA was performed to test for differences among the three groups in regards to total solid concentrations and average daily BW gains at each time point. A value of $P < 0.05$ was considered significant.

A total of 184 foals completed the trial, with 18 foals dropping out of the study for reasons unrelated to the vaccination or EPE. No adverse effects of the vaccine or administration were detected; all foals tolerated the vaccination well and required only minimal restraint for vaccine administration. Disease incidence was calculated to be 1.9% in the study population, which was markedly lower than the expected 10%. Three of the 106 non-vaccinated foals and 1/96 vaccinated foals exhibited clinical signs of EPE and disease was confirmed using paired IPMA titers in all cases and positive fecal PCR results in three cases. The difference was not statistically significant between the two groups ($P = 0.35$). The VNTR profile for *L. intracellularis* from the feces of the three foals with PCR positive results was similar amongst the foals and different from swine-derived isolates (including the vaccine derived strain; data not shown).

Seventy-nine of the 96 vaccinated horses seroconverted following vaccine administration (82%) and 63/106 non-vaccinated horses seroconverted due to natural infection (59%). Positive serology due to natural exposure in the absence of clinical signs of EPE was interpreted as sub-clinical disease. Categorization of the foals based on IPMA serology resulted in three groups: vaccinated ($n = 96$), subclinical ($n = 63$) and unaffected animals ($n = 43$). The vaccinated group had a significantly higher daily BW gain only at one time point (day 90) when compared to the sub-clinical group ($P = 0.042$). Mean total solid concentrations were within reference ranges for all groups at all time points (Table 2). Total solid concentrations were markedly decreased in the clinically affected foals at the time of illness (Table 1).

The study failed to prove the hypotheses, since the expected 10% disease incidence did not occur in the study population in 2009. Since the overall incidence of EPE did not decrease in Central-Kentucky (personal communications, Kentucky State Diagnostic Laboratory), it was possible that the vaccine organisms shed by the vaccinated foals triggered a protective immune response in non-vaccinated foals as the groups were housed together. Previous

Table 2
Average daily bodyweight gains (ADG) and total solid concentrations (TS) in vaccinated and naturally exposed weanling foals to *L. intracellularis*.

			Day 30	Day 60	Day 90	Day 120
Vaccinated group ($n = 96$)	ADG (kg/day)	Mean	2.337	1.549	1.932*	1.28
		Median	2.3	1.5	2.025	1.235
		SD	0.59	0.492	0.377	0.639
	TP (g/dL)	Mean	6.168*	6	5.981	6.177
		Median	6.2	6.067	6	6
		SD	0.273	0.304	0.357	0.318
Subclinical group ($n = 63$)	ADG (kg/day)	Mean	1.923	1.826	1.658	1.422
		Median	2.051	1.817	1.73	1.375
		SD	0.745	0.443	0.593	0.783
	TP (g/dL)	Mean	5.666	5.973	5.885	6.178
		Median	6	6	5.9	6.2
		SD	1.063	0.282	0.505	0.34
Unaffected group ($n = 43$)	ADG (kg/day)	Mean	2.239	1.375	1.867	1.845
		Median	2.121	1.463	1.806	1.826
		SD	0.658	0.628	0.54	0.507
	TP (g/dL)	Mean	6.116	6.112	6.046	6.219
		Median	6.1	6.1	6	6.2
		SD	3.111	0.304	0.206	0.322

SD, standard deviation; ADG, average daily bodyweight gain.
* $P < 0.05$ between groups.

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studies have shown that vaccinated foals shed *L. intracellularis* in their feces as a result of colonization of the intestinal tract by the vaccine strain (Pusterla et al., 2009a). Further, we speculated that vaccination of 50% of the foal population may have decreased environmental contamination by decreasing the number of foals shedding *L. intracellularis* following natural exposure. Further studies are warranted to prove efficacy by using larger sample sizes and geographically separating the vaccinated and control foals.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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